

Molecular evidence for the paraphyly of *Pseudancistrus sensu lato* (Siluriformes, Loricariidae), with revalidation of several genera

by

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ABSTRACT. - The genus *Pseudancistrus* is widely distributed in rocky, fast-flowing habitats of rivers draining all sides of the Guiana Shield and the northern slope of the Brazilian Shield. Initially diagnosed as having an “interoperculum” weakly evertible and lacking elongate odontodes, the definition of *Pseudancistrus* was subsequently expanded to include species with long, evertible cheek odontodes. Within *Pseudancistrus sensu lato* were included the already available genera *Guyanancistrus* and *Lithoxancistrus*. To evaluate the relevance of such a grouping, and particularly the morphology-based hypotheses of Armbruster (2004a, 2008), we reconstructed a phylogeny using mitochondrial and nuclear data and samples from 13 species currently included in *Pseudancistrus sensu lato*. All phylogenetic reconstructions recovered *Pseudancistrus sensu lato* as a paraphyletic assemblage of five unrelated lineages, and most of the alternative hypotheses evaluating monophyly of the genus were significantly rejected. Two of the recovered lineages were consistent with the genera *Guyanancistrus* and *Lithoxancistrus*, which are accordingly revalidated. A fourth lineage consisted of *Pseudancistrus pectegenitor* and *P. sidereus*, which may represent an undescribed genus. However their placement within *Lithoxancistrus* cannot be excluded. The fifth lineage consisted only of *P. genisetiger*, which was recovered as sister to *Hemipsilichthys gobio* and may represent an undescribed genus within the Delturinae.

RÉSUMÉ. - Évidences moléculaires de la paraphylie de *Pseudancistrus sensu lato* (Siluriformes, Loricariidae), avec revalidation de plusieurs genres.

Le genre *Pseudancistrus* est largement distribué dans les habitats rocheux et à eaux vives des rivières drainant l'ensemble du bouclier guyanais et le versant nord du bouclier brésilien. Initialement défini par un “interoperculum” pouvant être faiblement érigé et ne portant pas de longs odontodes, la définition de *Pseudancistrus* a par la suite été étendue afin d'y inclure des espèces aux odontodes jugaux longs et pouvant être érigés. Les genres *Guyanancistrus* et *Lithoxancistrus* ont ainsi été mis en synonymie de *Pseudancistrus*. Afin d'évaluer la pertinence de tels regroupements, et en particulier les hypothèses morphologiques proposées par Armbruster (2004a, 2008), nous avons reconstruit une phylogénie utilisant des données mitochondriales et nucléaires et incluant un échantillon de 13 espèces actuellement placées dans *Pseudancistrus sensu lato*. Toutes les reconstructions phylogénétiques retrouvent *Pseudancistrus sensu lato* en tant que groupe paraphylétique formé de cinq lignées distinctes, et la plupart des hypothèses alternatives évaluant la monophylie du genre ont été significativement rejetées. Deux des lignées retrouvées sont congruentes avec les genres *Guyanancistrus* et *Lithoxancistrus* qui sont en conséquence revalidés. Une quatrième lignée comprend les espèces *Pseudancistrus pectegenitor* et *P. sidereus* qui pourraient constituer un nouveau genre. Néanmoins, leur placement au sein de *Lithoxancistrus* ne peut pas être écarté. La cinquième lignée comprend uniquement *P. genisetiger* retrouvé en position soeur d'*Hemipsilichthys gobio* et qui pourrait former un nouveau genre au sein des Delturinae.

Key words. - Molecular phylogeny - Mitochondrial and nuclear genes - Topological tests - *Guyanancistrus* - *Lithoxancistrus* - Guianas.

Pseudancistrus currently encompasses 17 valid and several undescribed species of Loricariidae with a center of distribution on the Guiana and Brazilian Shields, in Northern South America. Bleeker described the genus *Pseudancistrus* in 1862 (in Bleeker, 1862-1863) and designated *Hypostomus barbatus* Valenciennes, 1840, as the type species. He distinguished the new genus from all other genera of his Plecostomini (Hypostominae with an adipose fin), except *Hypostomus* Lacépède, 1803, by having a weakly evertible “interoperculum” and lacking elongate bristly spines (odontodes). A snout margin bearing bristles from the tip to the branchial

opening and an unplated abdomen were the main characteristics distinguishing *Pseudancistrus* from *Hypostomus*.

Subsequent authors referred additional species corresponding to this diagnosis to *Pseudancistrus*. Also included were several species with long and evertible cheek odontodes, including the future type species of *Cordylancistrus* Isbrücker, 1980 and of *Dolichancistrus* Isbrücker, 1980. In his classification of Loricariidae, Isbrücker (1980) restricted *Pseudancistrus* to species with hypertrophied odontodes on sides of head and non-evertible (or very weakly evertible) cheek plates, consistent with the original definition of the

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genus. He placed the genus in the subfamily Hypostominae (a group largely corresponding with tribe Hypostomini of Armbruster, 2004a) based on the cheek plate character, despite having previously expressed strong doubts about this placement (Isbrücker, 1979). In a phylogenetic study of osteology, Schaefer (1986) transferred *Pseudancistrus* to subfamily Ancistrinae (largely corresponding with tribe Ancistrini of Armbruster, 2004a) based on it having a sickle-shaped opercle and despite the absence of evertible cheek plates. Later, Isbrücker *et al.* (1988) described the genus *Lithoxancistrus* for *L. orinoco*, which was characterized as similar to *Pseudancistrus* but with a tuft of long evertible cheek odontodes and three large buccal papillae (including two on dentaries). Isbrücker (*in* Isbrücker *et al.*, 2001) described *Guyanancistrus* to accommodate species that had been placed in *Lasiancistrus* Regan 1904 but were missing the characteristic whisker-like cheek odontodes of the latter, designating the Guianese *L. brevispinis* Heitmans *et al.*, 1983 as the type species.

Authors generally followed Isbrücker's (1980, 2001) definition of *Pseudancistrus* (e.g., Ouboter and Mol, 1993;

Le Bail *et al.*, 2000; Fisch-Muller, 2003), until Armbruster (2004a) included several additional taxa in the genus based on a phylogenetic analysis of morphological characters inclusive of most genera then in subfamilies Hypostominae and Ancistrinae. Armbruster (2004a, 2004b) placed in synonymy with *Pseudancistrus* two genera that have evertible cheek plates supporting hypertrophied odontodes (*Lithoxancistrus* and *Guyanancistrus*) transferred *Hemiancistrus megacephalus* (Günther, 1868) to *Pseudancistrus*, and added the new species *P. sidereus*. This revised *Pseudancistrus* was found to be monophyletic (Armbruster 2004a, 2008, see Fig. 1). Despite the lack of a single diagnostic character for *Pseudancistrus*, Armbruster (2004a) diagnosed the genus using a unique combination of six homoplastic synapomorphies: the loss of a suture between the pterotic-supracleithrum and hyomandibular (character 34: state 0), the loss of contact between the hyomandibular and prootic (35:1), a spoon-shaped anterior process of the metapterygoid (58:1), a thin nasal bone (105:0), a sphenotic that does not contact the posteriormost infraorbital (117:1), and a short ventral ridge of the basipterygium (172:1). The species with hypertrophied odontodes

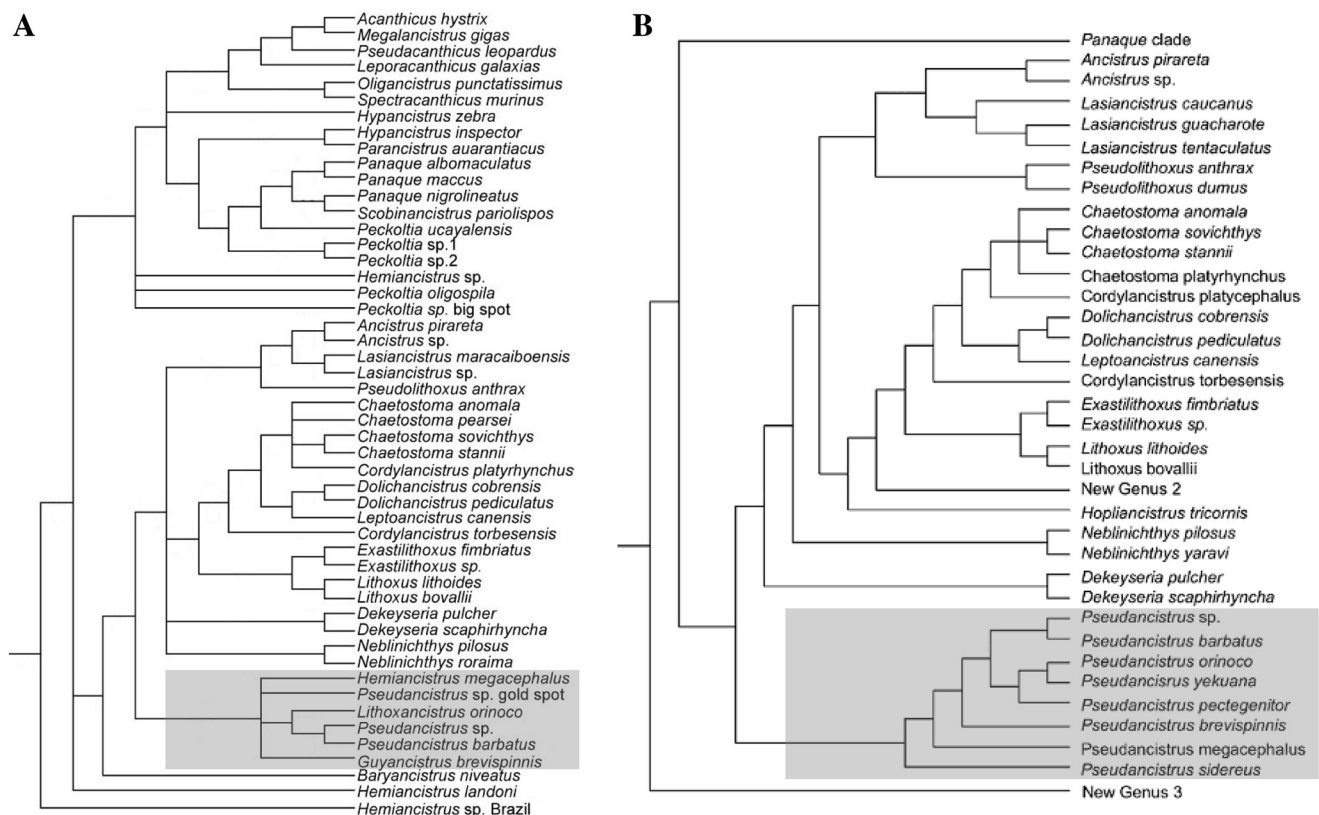


Figure 1. - Alternative phylogenetic hypotheses available in the literature. **A:** Hypothesis from Armbruster (2004a) representing the relationships of the taxa within the Ancistrini based on a Maximum Parsimony ordered analysis of 215 morphological characters: part of the strict consensus of 5098 most parsimonious trees of 1328 steps, CI = 0.203; based on this result, Armbruster (2004a) placed *Guyanancistrus*, *Lithoxancistrus* and *Hemiancistrus megacephalus* within *Pseudancistrus*. **B:** Hypothesis from Armbruster (2008) representing the relationships of the *Ancistrus* clade of the Ancistrini: part of the strict consensus of 1148 trees of 1388 steps, CI = 0.19, RI = 0.75. In both trees, species now placed in *Pseudancistrus* are shaded.

along the snout margin and non-evertible cheek plates (corresponding to previous diagnosis of *Pseudancistrus*) were considered as derived within *Pseudancistrus*. Armbruster and Taphorn (2008) and Lujan and Armbruster (2011) referred to this group of species having only limited ability to evert the cheek plates, with the addition of *P. genisetiger* Fowler, 1941, *P. papariae* Fowler, 1941 and *P. reus* Armbruster & Taphorn, 2008, as *Pseudancistrus sensu stricto*. This more restricted group is mostly distributed in coastal rivers of Northern South America from the Caroni River, a tributary of Lower Orinoco, to the Oyapock River (exceptions being *P. genisetiger* and *P. papariae*, which are found in coastal drainages of Northeastern Brazil). Based on a molecular analysis of the mitochondrial D-loop of select species from the Guianas, de Chambrier and Montoya-Burgos (2008) defined the *Pseudancistrus barbatus* group (referred to herein as the true *Pseudancistrus*), including four species: *P. barbatus*, *P. depressus* (Günther, 1968), *P. nigrescens* Eigenmann, 1912, and the newly described species *P. corantijnensis*.

Following Armbruster's greatly enlarged definition of the genus, *Pseudancistrus* currently includes 17 species, several of which have been recently described (Lujan *et al.*, 2007; Armbruster and Taphorn, 2008; de Chambrier and Montoya-Burgos, 2008; Willink *et al.*, 2010). *Pseudancistrus sensu lato* lacks externally diagnostic characters and includes species with all possible combinations of the snout and cheek odontode characteristics historically diagnostic for *Pseudancistrus*, *Lithoxancistrus*, and *Guyanancistrus*. To test the phylogenetic relationships hypothesized by Armbruster (2004a, 2008) and the monophyly of current taxonomic groupings, we herein conduct a phylogenetic analysis of two mitochondrial and one nuclear genes from 37 loricariid species (13 putative members of *Pseudancistrus sensu lato*, 18 species in 7 other Ancistrini genera, and 6 species from different genera outside Ancistrini).

MATERIAL AND METHODS

Taxonomic sampling

The molecular phylogeny was reconstructed for 42 specimens of the Hypostominae, which were selected to maximize representation of both *Pseudancistrus sensu lato* species and Ancistrini genera recovered as closely related in the morphological phylogenies of Armbruster (2004, 2008; Tab. I). The outgroup consisted of one species (*Hemiancistrus medians*) from the *Panaque* clade (forming the sister group of the *Ancistrus* clade according to Armbruster, 2008), four species of *Hypostomus* from the Hypostomini (forming the sister group of the Ancistrini and the Pterygoplichthyini according to Armbruster, 2004a, 2008), one species (*Harttia guianensis*) from the Loricariinae (forming the sister subfamily of the Hypostominae following results of Montoya-

Burgos *et al.*, 1998; Tab. I), one species (*Hemipsilichthys gobio*) from the Delturinae (forming the sister subfamily of all other Loricariidae following results of Montoya-Burgos *et al.*, 1998; Armbruster, 2004a, 2008; Tab. I), and one Callichthyidae (*Corydoras oiapoquensis*). The analyzed samples came from the tissue collections of the Muséum d'histoire naturelle de la ville de Genève (MHNG), Geneva, the Auburn University (AUM), and the Smithsonian Tropical Research Institute (STRI), Panama. The sequences were deposited in GenBank.

DNA extraction, amplification and sequencing

Tissue samples were preserved in 80% ethanol and stored at -20°C. Total genomic DNA was extracted with the DNeasy Tissue Kit (Qiagen) following the instructions of the manufacturer. The PCR amplifications of mitochondrial 12S and 16S, and the nuclear Fish Reticulon-4 (F-RTN4) genes were carried out using the Taq PCR Core Kit (Qiagen). The methodology for PCR amplifications followed Covain *et al.* (2008) for partial 12S and 16S, and Chiacchio *et al.* (2008) for F-RTN4. To amplify the complete 12S gene, two additional primers were used: An12S-1D (Montoya-Burgos, unpubl. data), and H1478 (Kocher *et al.*, 1989). The amplifications were performed in a total volume of 50 µl, containing 5 µl of 10x reaction buffer, 1 µl of dNTP mix at 10 mM each, 1 µl of each primer at 10 µM, 0.2 µl of Taq DNA Polymerase equivalent to 1 unit of Polymerase per tube, and 1 to 4 µl of DNA. Cycles of amplification were programmed with the following profile: (1) 3 min. at 94°C (initial denaturing), (2) 35 sec. at 94°C, (3) 30 sec. at 52-54°C, (4) 80 sec. at 72°C, and (5) 5 min. at 72°C (final elongation). Steps 2 to 4 were repeated 35 to 39 times according to the quality and concentration of DNA. PCR products were purified with the High Pure PCR Product Purification Kit (Roche). Sequencing reactions were performed with the Big Dye Terminator Cycle Sequencing Ready Reaction 3.1 Kit (Applied Biosystems) following instructions of the manufacturer, and were loaded on an automatic sequencer 3100-Avant Genetic Analyzer (Applied Biosystems, Perkin-Elmer).

Sequence alignment, phylogenetic reconstructions, and topological tests

The DNA sequences were edited and assembled with BioEdit 7.0.1 (Hall, 1999), and aligned using ClustalW (Thompson *et al.*, 1994) using default parameters. The resulting alignment was optimized by eye and ambiguously aligned positions were removed. Since mitochondrial DNA is presumably transmitted through maternal lineage as a single non-recombining genetic unit (Meyer, 1993), we created a first partition corresponding to the mitochondrial genes. As mutational patterns in F-RTN4 are characterized by insertions/deletions in the introns, and transitions/transversions in exons, we created two additional partitions for this nuclear

Table I. - Taxa list, specimen and sequence data analyzed in this study (n = 45, including 18 representatives of *Pseudancistrus sensu lato*). Institutional acronyms follow Fricke and Eschmeyer (2010). 1 outgroup, 2 according to the exporter, 3 aquarium specimen, 4 holotype, 5 paratype, 6 *Guyanancistrus sensu* Isbrücker, 2001, 7 *Lithoxancistrus sensu* Isbrücker, 2001

Species	Catalog Number	Field Number	Locality
Callichthyidae			
<i>Corydoras oiapoquensis</i> ¹	MHNG 2682.023	GF06-186	French Guiana, Oyapock River
Loricariidae			
Delturinae			
<i>Hemipsilichthys gobio</i> ¹	LBP 2368	15363	Brazil, Rio Macaquinho
Loricariinae			
<i>Harttia guianensis</i> ¹	MHNG 2643.016	GF00-351	French Guiana, Marouini River
Hypostominae			
Hypostomini			
<i>Hypostomus plecostomoides</i> ¹	NA	VZ 58	Venezuela, Rio Orinoco
<i>Hypostomus</i> sp. Monzon ¹	MHNG 2721.062	PE08-198	Peru, Rio Monzon
<i>Hypostomus boulengeri</i> ¹	MHNG 2519.23	ASU7	Paraguay, Rio Paraguay
<i>Hypostomus gymnorhynchus</i> ¹	MHNG 2621.098	SU01-160	French Guiana, Approuague River
Ancistrini			
<i>Ancistrus cirrhosus</i> ¹	MHNG 2645.037	MUS 202	Argentina, Rio Uruguay
<i>Dekeyseria picta</i> ³	MHNG 2588.046	MUS 162	Brazil, Rio Negro ²
<i>Dekeyseria pulchra</i> ³	MHNG 2677.062	MUS 176	Colombia, Rio Orinoco ²
<i>Dekeyseria scaphirhyncha</i>	AUM 43874	V5528	Venezuela, Rio Orinoco
<i>Hemiancistrus medians</i> ¹	MHNG 2664.078	GF00-084	French Guiana, Marouini River
<i>Hopliancistrus tricornis</i> ³	MHNG 2588.051	MUS 146	Brazil, Rio Xingu ²
<i>Lasiancistrus</i> aff. <i>caucanus</i>	MHNG 2586.043	MUS 118	Colombia, Rio Cesar
<i>Lasiancistrus heteracanthus</i>	MHNG 2613.037	CA 013	Peru, Rio Pauya
<i>Lasiancistrus planiceps</i>	STRI-01805	Stri 3526	Panama, Rio Tuira
<i>Lasiancistrus saetiger</i>	MHNG 2602.016	BR98-148	Brazil, Rio Guamá
<i>Lasiancistrus schomburgkii</i> ⁶	MHNG 2651.009	PE08-719	Peru, Rio Cushabatay
<i>Lasiancistrus schomburgkii</i> ⁶	MHNG 2651.068	GY04-308	Guyana, Essequibo River
<i>Lasiancistrus schomburgkii</i> ⁶	MHNG 2710.055	PE08-277	Peru, Rio Pucayacu
<i>Lasiancistrus tentaculatus</i> ³	MHNG uncat.	MUS 573	Colombia, Rio Orinoco ²
<i>Lithoxus lithoides</i>	MHNG 2651.087	GY04-136	Guyana, Rupununi River
<i>Lithoxus pallidimaculatus</i>	MHNG 2621.066	SU01-096	Suriname, Suriname River
<i>Lithoxus planquettei</i>	MHNG 2722.060	GF03-055	French Guiana, Orapu River
<i>Pseudancistrus barbatus</i>	MHNG 2653.059	GF00-074	French Guiana, Maroni River
<i>Pseudancistrus brevispinis</i> ⁶	MHNG 2725.099	GF00-103	French Guiana, Tampoc River
<i>Pseudancistrus brevispinis</i> ⁶	MHNG 2621.073	SU01-121	Suriname, Nickerie River
<i>Pseudancistrus corantijnensis</i> ⁴	MHNG 2672.092	SU05-296	Suriname, Corantijne River
<i>Pseudancistrus depressus</i>	MHNG 2674.026	SU05-020	Suriname, Suriname River
<i>Pseudancistrus genisetiger</i> ⁷	MHNG 2593.061	MUS 173	Brazil, Recife
<i>Pseudancistrus longispinis</i> ⁶	MHNG 2725.100	GF99-204	French Guiana, Oyapock River
<i>Pseudancistrus niger</i> ⁶	MHNG 2722.089	GF99-185	French Guiana, Oyapock River
<i>Pseudancistrus nigrescens</i>	MHNG 2651.069	GY04-313	Guyana, Essequibo River
<i>Pseudancistrus nigrescens</i>	MHNG 2650.087	GY04-260	Guyana, Essequibo River
<i>Pseudancistrus orinoco</i> ⁷	AUM 43725	V5246	Venezuela, Rio Casiquiare
<i>Pseudancistrus orinoco</i> ⁷	AUM 42179	P4527	Venezuela, Rio Casiquiare
<i>Pseudancistrus pectegenitor</i> ⁵	AUM 42202	V5363	Venezuela, Rio Casiquiare
<i>Pseudancistrus pectegenitor</i> ⁵	ANSP 182801 (ex AUM 42181)	V5433	Venezuela, Rio Orinoco
<i>Pseudancistrus sidereus</i>	AUM 43443	P4871	Venezuela, Rio Casiquiare
<i>Pseudancistrus sidereus</i>	AUM 42180	P4537	Venezuela, Rio Casiquiare
<i>Pseudancistrus</i> sp. L17	MHNG 2586.046	MUS 132	Brazil, Rio Xingu
<i>Pseudancistrus</i> sp. Nassau ⁶	MHNG 2679.099	MUS 300	Suriname, Nassau Mountains
<i>Pseudolithoxus</i> cf. <i>kelsorum</i> ³	MHNG 2679.043	MUS 260	Colombia, Rio Orinoco ²
<i>Pseudolithoxus dumus</i> ³	MHNG 2708.080	MUS 288	Colombia, Rio Orinoco ²
<i>Pseudolithoxus tigris</i>	AUM 42215	V5292	Venezuela, Rio Orinoco

Table I. - Continued.

Species	mt 12S+16S GenBank No.	Ref.	F-RTN4 GenBank No.	Ref.
Callichthyidae				
<i>Corydoras oiapoquensis</i> ¹	GU210260/GU210685	Alexandrou <i>et al.</i> , 2011	GU210997	Alexandrou <i>et al.</i> , 2011
Loricariidae				
Delturinae				
<i>Hemipsilichthys gobio</i> ¹	FJ965460/FJ434499	Chiachio <i>et al.</i> , 2008	EU817547	Chiachio <i>et al.</i> , 2008
Loricariinae				
<i>Harttia guianensis</i> ¹	EU310447	Covain <i>et al.</i> , 2008	FJ013232	Chiachio <i>et al.</i> , 2008
Hypostominae				
Hypostomini				
<i>Hypostomus plecostomoides</i> ¹	NA	NA	EU817561	Chiachio <i>et al.</i> , 2008
<i>Hypostomus</i> sp. Monzon ¹	JN855753	This study	JN855790	This study
<i>Hypostomus boulengeri</i> ¹	NA	NA	EU817560	Chiachio <i>et al.</i> , 2008
<i>Hypostomus gymnorhynchus</i> ¹	JN855752	This study	JN855789	This study
Ancistrini				
<i>Ancistrus cirrhosus</i> ¹	EU310442	Covain <i>et al.</i> , 2008	HM623638	Rodriguez <i>et al.</i> , 2011
<i>Dekeyseria picta</i> ³	JN855716	This study	JN855755	This study
<i>Dekeyseria pulchra</i> ³	JN855718	This study	NA	This study
<i>Dekeyseria scaphirhyncha</i>	JN855717	This study	JN855756	This study
<i>Hemiancistrus medians</i> ¹	JN855719	This study	JF747011	Fisch-Muller <i>et al.</i> , 2012
<i>Hoplancistrus tricornis</i> ³	JN855728	This study	JN855765	This study
<i>Lasiancistrus</i> aff. <i>caucanus</i>	JN855749	This study	JN855786	This study
<i>Lasiancistrus heteracanthus</i>	JN855750	This study	JN855787	This study
<i>Lasiancistrus planiceps</i>	JN855748	This study	JN855785	This study
<i>Lasiancistrus saetiger</i>	JN855715	This study	JN855754	This study
<i>Lasiancistrus schomburgkii</i> ⁶	JN855745	This study	JN855782	This study
<i>Lasiancistrus schomburgkii</i> ⁶	JN855746	This study	JN855783	This study
<i>Lasiancistrus schomburgkii</i> ⁶	JN855747	This study	JN855784	This study
<i>Lasiancistrus tentaculatus</i> ³	JN855751	This study	JN855788	This study
<i>Lithoxus lithoides</i>	JN855740	This study	JN855777	This study
<i>Lithoxus pallidimaculatus</i>	JN855741	This study	JN855778	This study
<i>Lithoxus planquettei</i>	JN855742	This study	JN855779	This study
<i>Pseudancistrus barbatus</i>	JN855724	This study	JN855761	This study
<i>Pseudancistrus brevispinis</i> ⁶	JN855735	This study	JN855772	This study
<i>Pseudancistrus brevispinis</i> ⁶	JN855736	This study	JN855773	This study
<i>Pseudancistrus corantijnensis</i> ⁴	JN855744	This study	JN855781	This study
<i>Pseudancistrus depressus</i>	JN855743	This study	JN855780	This study
<i>Pseudancistrus genisetiger</i> ⁷	JN855727	This study	JN855764	This study
<i>Pseudancistrus longispinis</i> ⁶	JN855720	This study	JN855757	This study
<i>Pseudancistrus niger</i> ⁶	JN855722	This study	JN855759	This study
<i>Pseudancistrus nigrescens</i>	JN855733	This study	JN855770	This study
<i>Pseudancistrus nigrescens</i>	JN855734	This study	JN855771	This study
<i>Pseudancistrus orinoco</i> ⁷	JN855729	This study	JN855766	This study
<i>Pseudancistrus orinoco</i> ⁷	JN855730	This study	JN855767	This study
<i>Pseudancistrus pectegenitor</i> ⁵	JN855732	This study	JN855769	This study
<i>Pseudancistrus pectegenitor</i> ⁵	JN855731	This study	JN855768	This study
<i>Pseudancistrus sidereus</i>	JN855738	This study	JN855775	This study
<i>Pseudancistrus sidereus</i>	JN855739	This study	JN855776	This study
<i>Pseudancistrus</i> sp. L17	JN855726	This study	JN855763	This study
<i>Pseudancistrus</i> sp. Nassau ⁶	JN855737	This study	JN855774	This study
<i>Pseudolithoxus</i> cf. <i>kelsorum</i> ³	JN855725	This study	JN855762	This study
<i>Pseudolithoxus dumus</i> ³	JN855723	This study	JN855760	This study
<i>Pseudolithoxus tigris</i>	JN855721	This study	JN855758	This study

gene fragment. Combinability between mitochondrial and nuclear markers was secondarily assessed using the Congruence Among Distance Matrices (CADM) test (Legendre and Lapointe, 2004) as implemented in ape 2.7.1 (Paradis *et al.*, 2004; Paradis, 2006) in R 2.12.1 (R Development Core Team, 2009). The CADM test is a generalization to several distance matrices of the Mantel test (Mantel, 1967), and is used to test against the incongruence of all distance matrices. Pairwise maximum likelihood (ML; Felsenstein, 1981) distances were computed with Treefinder (Jobb *et al.*, 2004; version of October 2008) for each partition using a likelihood model under which the pairwise distances are optimized. Appropriate substitution models corresponding to each potential partition were accordingly estimated with the corrected Akaike Information Criterion (Sugiura, 1978) as implemented in Treefinder. The CADM test was computed using 9,999 permutations of the three ML distances matrices. Two phylogenetic reconstruction methods allowing the analysis of partitioned data were used. In each reconstruction method gaps were considered as missing data, and bases in missing fragments were coded as ambiguities (N). First, a ML reconstruction was performed with Treefinder, and robustness of the results was estimated by resampling the data set with the nonparametric bootstrap (Efron, 1979) following Felsenstein's (1985) methodology with 1,000 pseudoreplicates. Second, a Bayesian inference analysis was conducted in MrBayes 3.1.2 (Huelsenbeck and Ronquist, 2001; Ronquist and Huelsenbeck, 2003). Two runs of four chains (one cold, three heated) were conducted simultaneously for 2×10^7 generations, with the tree space sampled each 100th generation. After a visual representation of the evolution of the likelihood scores, and checking for the stationarity of all model parameters using Tracer 1.5 [Rambaut and Drummond, 2007; i.e., potential scale reduction factor (PSRF), uncorrected roughly approached 1 as runs converged (Gelman and Rubin, 1992), and Effective Sample size (ESS) of all parameters superior to 200], the 5×10^5 first generations were discarded as burn-in. The remaining trees were used to compute the consensus tree. Intensive calculations such as bootstrapping and Bayesian inferences were performed on the TITAN cluster at the University of Oslo, Norway, through Biportal (Kumar *et al.*, 2009).

Alternative topologies were tested under the null hypothesis that all phylogenetic hypotheses (trees) provided equally good explanations of the data using the Shimodaira-Hasegawa (SH) test (Shimodaira and Hasegawa, 1999) and the Approximately Unbiased (AU) test (Shimodaira, 2002), with addition of the Expected-Likelihood Weights (ELW) of the alternative hypothesis (Strimmer and Rambaut, 2002) as implemented in Treefinder using 1×10^6 RELL replicates (Kishino *et al.*, 1990). The Expected-Likelihood Weights correspond to the relative frequency that an hypothesis has the best resampled likelihood score weighted by the model

selection probabilities. The Expected-Likelihood Weights can thus directly be interpreted as confidence in a given hypothesis. All alternative topologies were constructed in order to reflect, as much as possible given our taxonomic sampling, already proposed phylogenetic hypotheses (Armbruster 2004a, 2008 in figure 1), or different reconstructions evaluating the monophyly of *Pseudancistrus*. Alternative hypotheses are summarized in figure 3.

Finally, synapomorphic characters provided for *Pseudancistrus sensu lato* were submitted to the RUNS test (Sokal and Rohlf, 1995) following Abouheif's (1999) procedure. This test against phylogenetic independence allows the detection of self-similarities (i.e. autocorrelation) among adjacent observations. It is used to test whether observations (i.e. character states) in an ordered sequence (at tips of a phylogenetic tree) occur at random. The tree topology used was the best ML tree, and tip values corresponded to character states provided for the six synapomorphies defining *Pseudancistrus sensu lato* (Armbruster 2004a, 2008). For species differing between our sampling and those from the literature, and because these synapomorphies were used to define generic rank, the values of the different character states of a sister or closely related species (within a given genus) were used. In addition, we arbitrarily assigned the synapomorphic character states provided for *Pseudancistrus sensu lato* to *P. genisetiger*, and J.W. Armbruster (Auburn University) generously provided his data for *Hemiancistrus medians*. RUNS-mean were estimated after 9,999 random permutations of tips around nodes and compared to the randomized average statistics obtained after 9,999 random shuffling of tips (and permutations of tips around nodes) using Phylogenetic Independence version 2.0 (Reeve and Abouheif, 2003).

RESULTS

Phylogenetic analyses

We sequenced the almost complete 12S and 16S mitochondrial genes, and the partial nuclear gene F-RTN4 for 39 representatives of the Hypostominae. Sequences of *Harttia guianensis*, *Ancistrus cirrhosus*, *Hemipsilichthys gobio*, *Hypostomus boulengeri*, *Hp. plecostomoides*, and *Corydoras oiapoquensis* were obtained from GenBank using the accession numbers given in Covain *et al.* (2008), Chiachio *et al.* (2008), Alexandrou *et al.* (2011), Rodriguez *et al.* (2011), and Fisch-Muller *et al.* (2012). The final sequence alignment included 5,242 positions from which 2,401 corresponded to the mitochondrial genes, 886 to the exonic regions of the F-RTN4 gene, and 1,955 to the intronic regions of the F-RTN4 gene. No significant conflicting phylogenetic signal was detected in the data set since the global CADM test rejected the null hypothesis of incongruence between matrices (CADM: $W = 0.7403$, $\chi^2_{\text{ref}} = 2196.3883$,

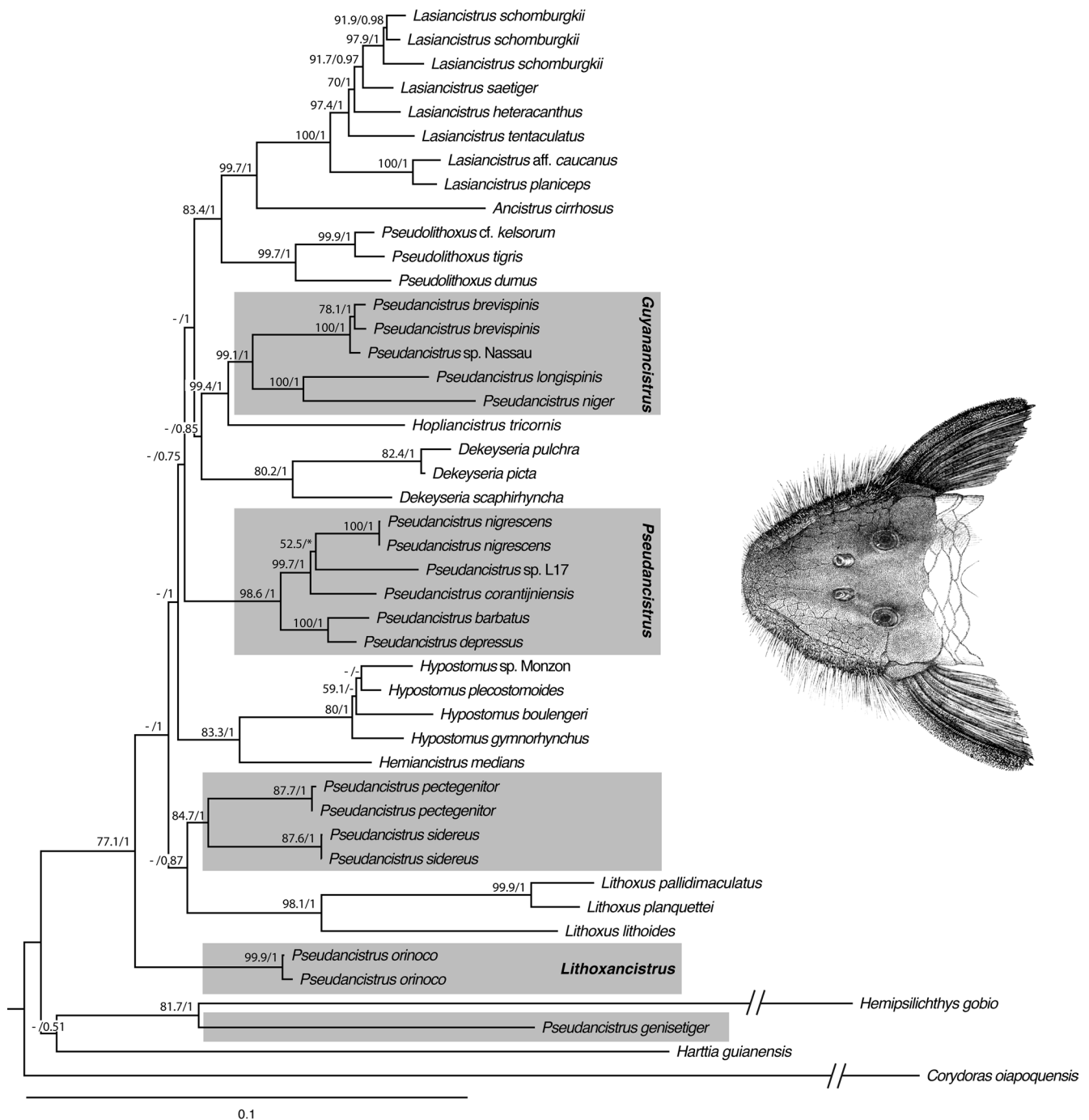


Figure 2. - Maximum likelihood tree of the Hypostominae including 18 *Pseudancistrus sensu lato* inferred from the analysis of partial mt 12S and 16S, and F-RTN4 nuclear gene sequences (-lnL = 27519.07). The best fit substitution models used were GTR + G for mitochondrial genes and intronic regions of F-RTN4, and J2 + G for exonic regions of F-RTN4. The GTR + G model was used for each of the three partitions for the Bayesian inference. Both reconstructions lead to equivalent tree topologies. Numbers above branches indicate bootstrap supports above 50 for the ML analysis followed by posterior probabilities above 0.5 for the Bayesian inference. Sign (-) indicates values below 50 % bootstrap leading to polytomies in the ML bootstrap majority rule consensus tree (consensus level = 50). Sign * indicates incongruence between the two trees. *Pseudancistrus* lineages are shaded, and bold style refers to names already available for several lineages. Drawing represents the head of a male *P. barbatus* bearing sexually dimorphic enlarged snout odontodes; reprinted from Darwin (1871).

$p(\chi^2_{\text{ref}} \geq \chi^2_*) = 0.0001$). The CADM *a posteriori* tests did not detect any conflicting matrix in the data (\bar{r}_s mitochondrion

$= 0.4864$, $p(\bar{r}_{s \text{ ref}} \geq \bar{r}_s^*) = 0.0003$; \bar{r}_s exons $= 0.6758$, $p(\bar{r}_{s \text{ ref}} \geq \bar{r}_s^*) = 0.0003$; \bar{r}_s introns $= 0.6690$, $p(\bar{r}_{s \text{ ref}} \geq \bar{r}_s^*) = 0.0003$).

The sequences were consequently concatenated, and three partitions corresponding to mitochondrial genes, exonic parts of F-RTN4, and intronic parts of F-RTN4 were used to reconstruct the trees. The models GTR + G (Tavaré, 1986) for mitochondrial genes and intronic regions of F-RTN4, and J2 + G (Jobb *et al.*, 2004) for exonic regions of F-RTN4 displayed the smallest AICc and fit our data best as indicated by Treefinder. The GTR + G model was used for each of the three partitions for the Bayesian inference, with each partition assigned its own among-sites heterogeneity rate.

Rooting of the resulting trees was problematic since several of the expected outgroup species (members of the *Pan-aque* clade and Hypostomini) connected within the ingroup. We thus used *C. oiapoquensis* (family Callichthyidae) to polarize the tree. Bayesian and ML phylogenetic reconstructions lead to similar tree topologies. The best ML tree ($-\ln L = 27519.07$; Fig. 2) recovered the species *P. genisetiger* as a member of the outgroup in a sister position to *Hm. gobio* (subfamily Delturinae), a position strongly supported by both bootstrap support and Bayesian posterior probabilities. Within the ingroup, the first diverging group corresponded to *Pseudancistrus orinoco* (type species of *Lithoxancistrus*) as sister of the remaining Hypostominae. Within this group, the following lineages were split off in successively nested fashion: representatives of *Pseudancistrus* from the Orinoco drainage (*P. pectegenitor* and *P. sidereus*) with *Lithoxus* as sister groups, the four species of *Hypostomus* and *Hemiancistrus medians* as sister groups, and then the true *Pseudancistrus* including *P. barbatus* (type species of the genus). These true *Pseudancistrus* were sister to a group that was split into two sister lineages. The first consisted of *Dekeyseria* as sister to *Hopliancistrus tricornis* plus *Pseudancistrus longispinis*, *P. niger*, *P. sp. Nassau*, and *P. brevispinis* (type species of *Guyanancistrus*). The second lineage consisted of *Ancistrus* in a sister position to *Lasiancistrus*. Bayesian reconstruction differed insofar as *Pseudancistrus* sp. L17 was recovered as sister to *P. corantijniensis* with high statistical support (0.93). Bootstrap analysis provided high statistical support for all within and between closely related genera relationships, whereas deeper nodes were poorly supported (Fig. 2). Alternatively, posterior probabilities were high (> 0.7) all along the phylogeny, resulting in a fully resolved topology (consensus level 0.5). In both ML and Bayesian reconstructions, *Pseudancistrus* was always found paraphyletic and consisting of five lineages.

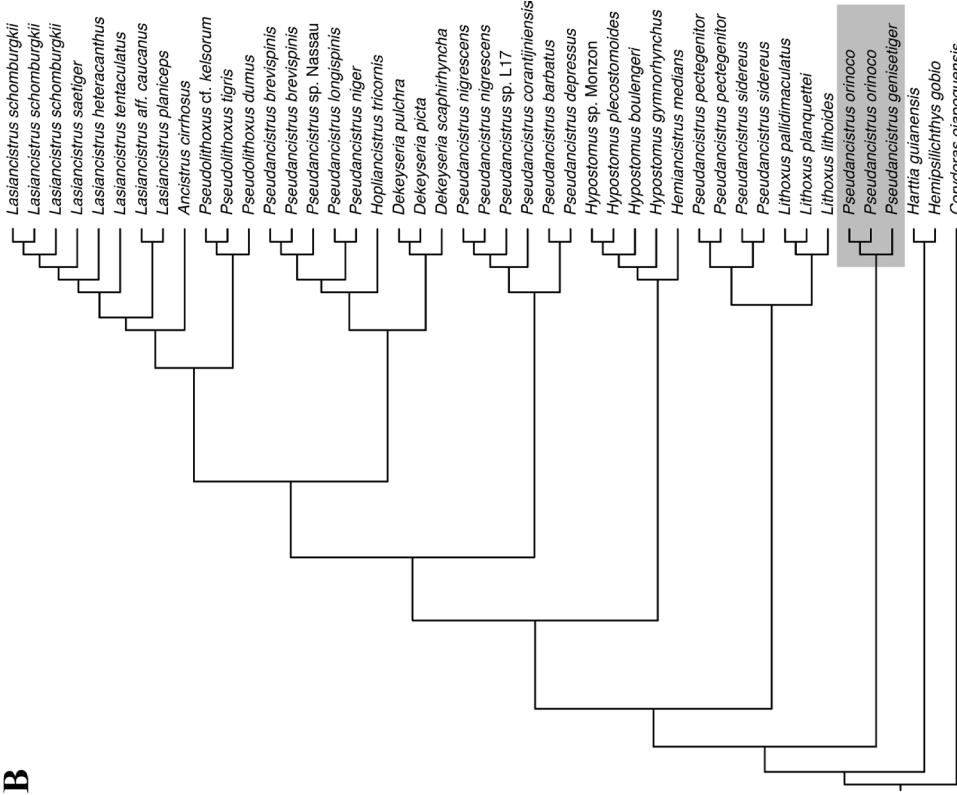
Topological tests

Alternative hypotheses concerning the monophyly of *Pseudancistrus sensu lato* were evaluated (Fig. 3). Prior to this evaluation, different hypotheses were tested to minimize differences between the best ML tree and the alternative reconstructed hypotheses, and to evaluate more widely accepted relationships. The sister relationship between

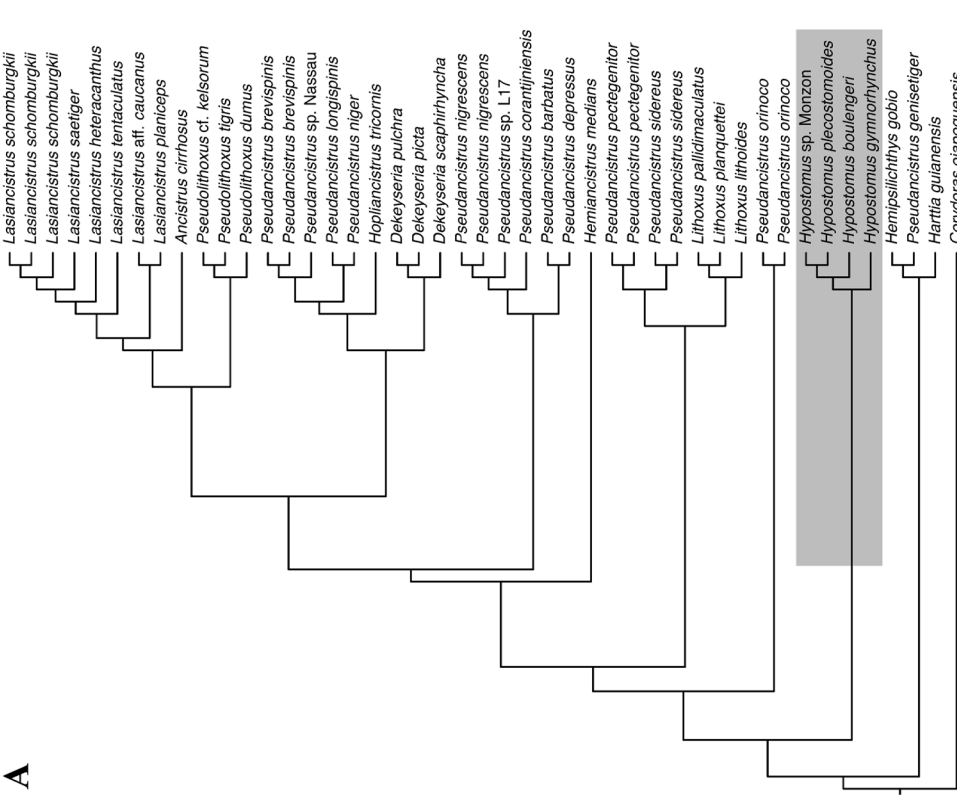
Hypostomini (represented by the four species of *Hypostomus*) and the Ancistrini (Fig. 3A) was significantly rejected by both AU and ELW tests, but not by the SH test. The placement of *P. genisetiger* as sister species of *P. orinoco* (type species of *Lithoxancistrus*) to evaluate whether *P. genisetiger* could be a member of *Lithoxancistrus* (following Isbrücker, 2001) was also significantly rejected by all tests but the SH test (Fig. 3B). In the same way, the placement of *P. genisetiger* as a member of the Hypostominae (but without *a priori* defined internal relationships) was also rejected (Fig. 3C). Consequently, alternative hypotheses concerning the monophyly of *Pseudancistrus sensu lato* were reconstructed, always excluding *P. genisetiger* due to its exclusion from the ingroup. The hypothesis proposed by Armbruster (2004a) consisting of a polytomy of all *Pseudancistrus* lineages (including *Lithoxancistrus* and *Guyanancistrus*), and with *Lithoxancistrus orinoco* as sister to the true *Pseudancistrus* (Figs 1A, 3D) was significantly rejected by all tests except the SH test. Refinement of the original hypothesis (Armbruster, 2004a) proposed by Armbruster (2008) featuring additional species from the Orinoco drainage (Figs 1B, 3E) was also significantly rejected given the data. The monophyly of *Pseudancistrus* excluding *Guyanancistrus* (Fig. 3F) was also significantly rejected by both AU and ELW tests but not by the SH test ($p = 0.454$). The placement of *Guyanancistrus* species within *Lasiancistrus* (following Heitmans *et al.*, 1983) but without *a priori* defined internal relationships was significantly rejected by all tests (Fig. 3G). The sister relationship between *P. orinoco* (type species of *Lithoxancistrus*) and the true *Pseudancistrus* (including *P. barbatus*, type species; Fig. 3H) was also significantly rejected by both the AU and ELW tests, but not by the SH test. The sister relationship between the true *Pseudancistrus* and species from the Orinoco drainage (excluding *P. orinoco*; Fig. 3I) was rejected by all tests except the SH test. Sister relationship between *P. orinoco* and other species from Orinoco drainage (Fig. 3J) was not rejected by any testing procedures ($0.158 \leq p \leq 0.965$).

Tests of phylogenetic autocorrelation

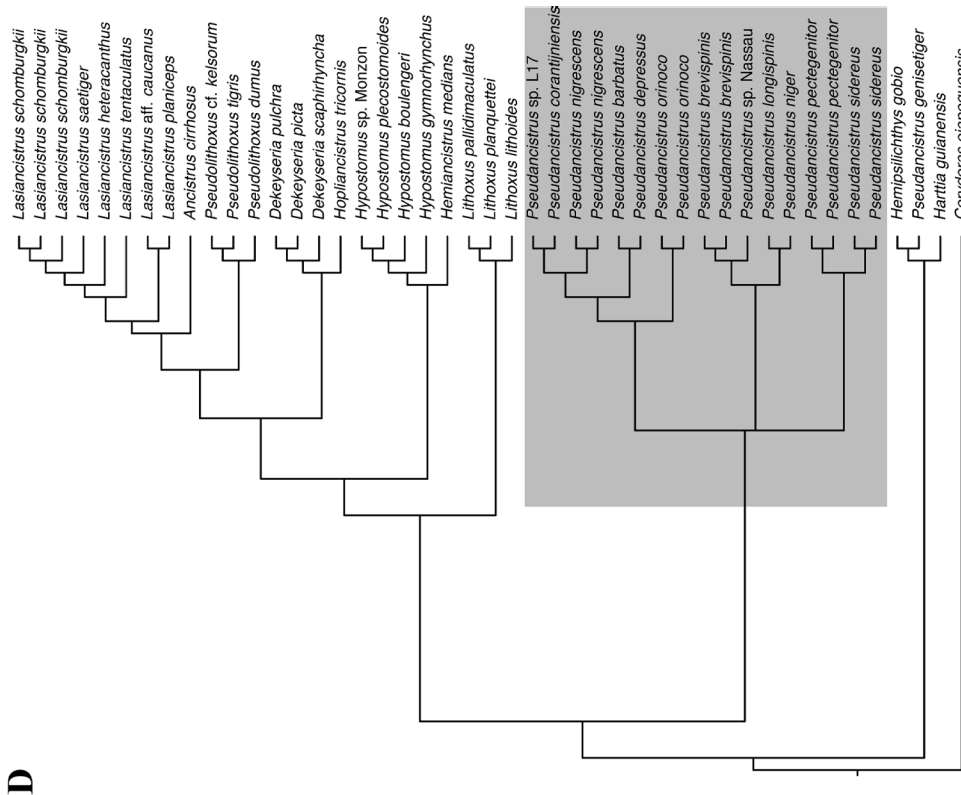
All the RUNS tests conducted on the six synapomorphies provided for *Pseudancistrus sensu lato* given the molecular phylogeny (Fig. 4) significantly rejected the null hypothesis of phylogenetic independence of the data. The presence or absence of a suture between the pterotic-supracleithrum and hyomandibular (character 34; RUNS-mean = 6.535; $p(X^* \leq X_{obs.}) = 0.0001$), the presence or absence of a contact between the hyomandibular and prootic (35; RUNS-mean = 8.378; $p(X^* \leq X_{obs.}) = 0.0001$), the characteristics of the anterior process of the metapterygoid (58; RUNS-mean = 10.718; $p(X^* \leq X_{obs.}) = 0.0001$), the size of the nasal bone (105; RUNS-mean = 12.392; $p(X^* \leq X_{obs.}) = 0.0001$), the presence or absence of an external contact between the sphenotic and



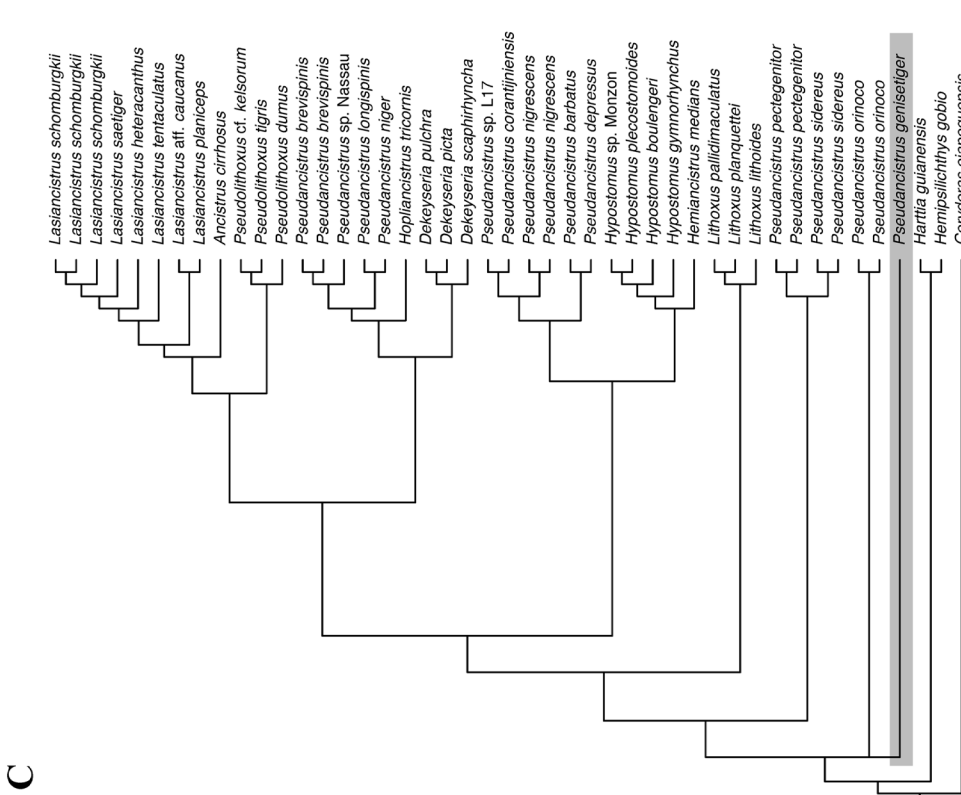
Hypothesis	lnL	Δ lnL	SH	AU	ELW
H0	-27519.07	-	-	-	-
H2	-27605.22	86.15	0.311	0.000	0.000



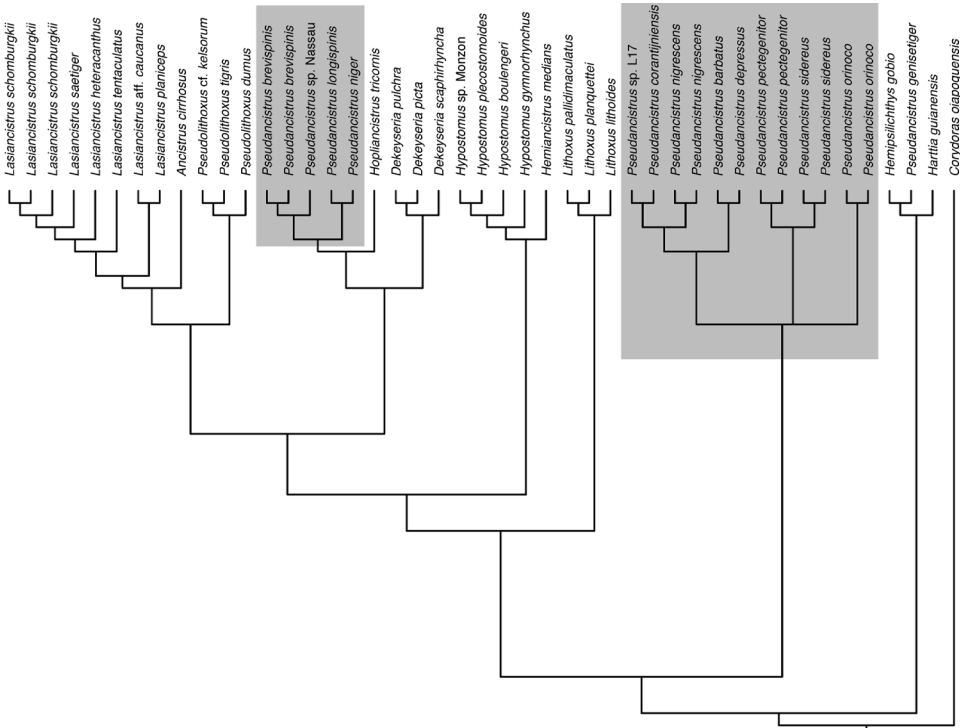
Hypothesis	lnL	Δ lnL	SH	AU	ELW
H0	-27519.07	-	-	-	-
H1	-27603.03	83.96	0.313	0.000	0.000



Hypothesis	lnL	Δ lnL	SH	AU	ELW
H0	-27519.07	-	-	-	-
H4	-27629.42	110.35	0.231	0.000	0.000

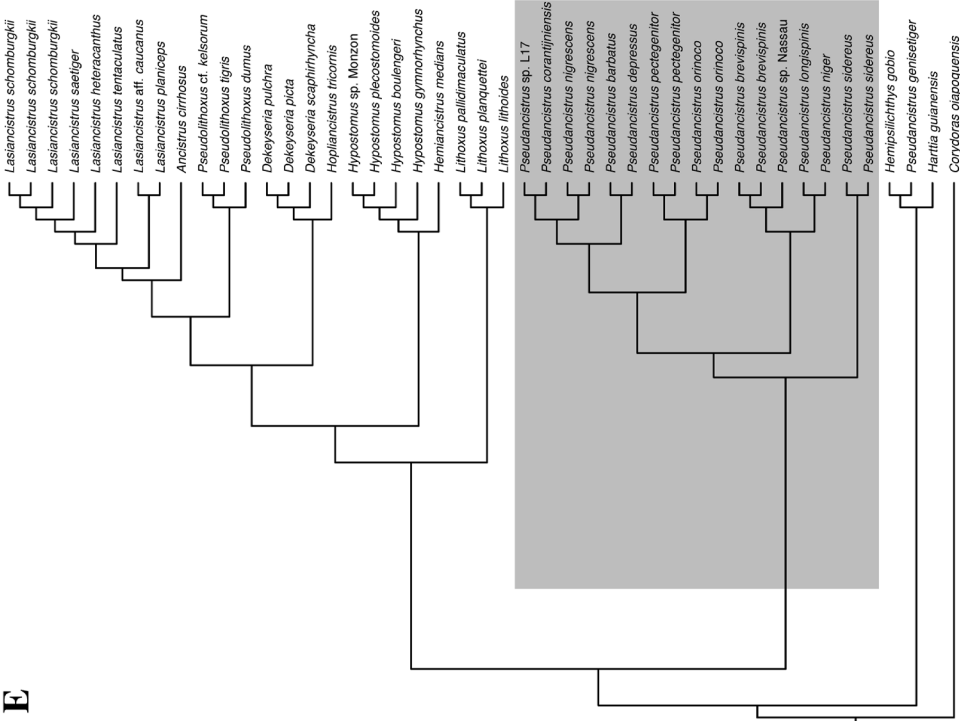


Hypothesis	lnL	Δ lnL	SH	AU	ELW
H0	-27519.07	-	-	-	-
H3	-27597.42	78.35	0.342	0.000	0.000



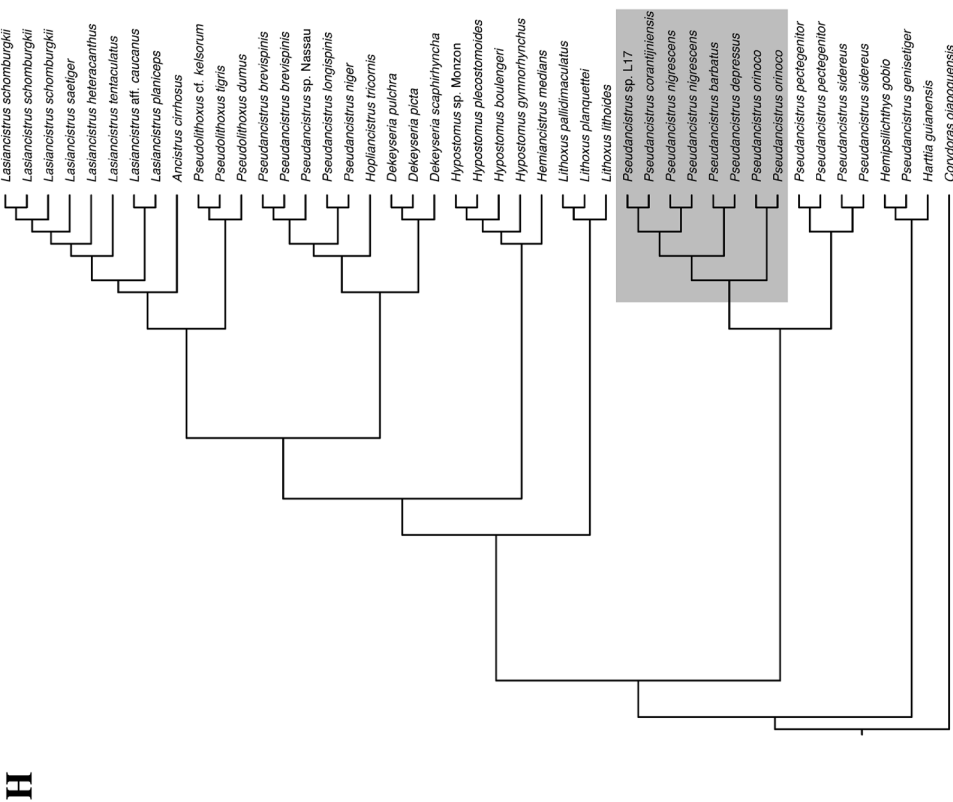
F

Hypothesis	lnL	$\Delta\ln L$	SH	AU	ELW
H0	-27519.07	-	-	-	-
H6	-27573.63	54.56	0.454	0.000	1.1E-06

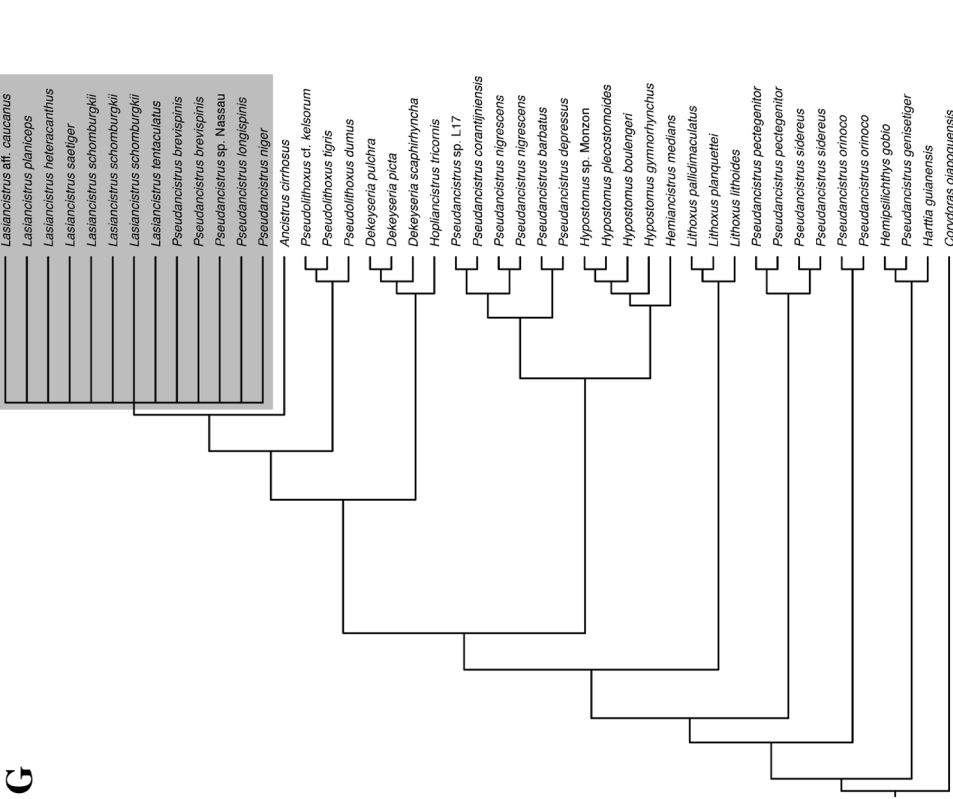


E

Hypothesis	lnL	$\Delta\ln L$	SH	AU	ELW
H0	-27519.07	-	-	-	-
H5	-27657.03	137.96	0.159	0.000	0.000



Hypothesis	lnL	Δ lnL	SH	AU	ELW
H0	-27519.07	-	-	-	-
H8	-27574.03	54.96	0.447	0.000	8.7E-07



Hypothesis	lnL	Δ lnL	SH	AU	ELW
H0	-27519.07	-	-	-	-
H7	-29720.83	2201.76	0.000	0.000	0.000

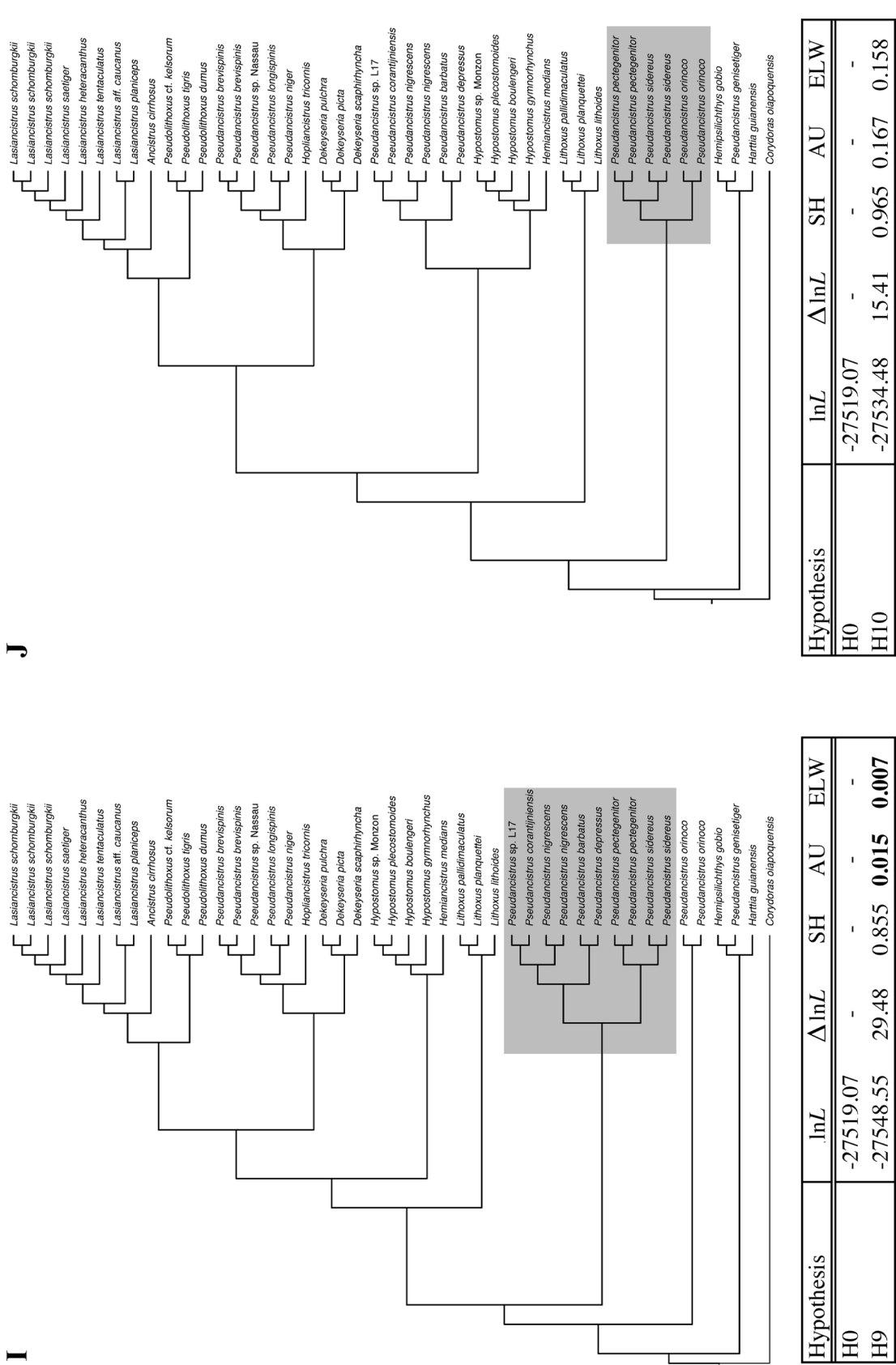


Figure 3. - Best topological representations of the alternative phylogenetic hypotheses evaluated using the Shimodaira and Hasegawa (SH), the Approximately Unbiased (AU) and the Expected-Likelihood Weights (ELW) testing procedures. lnL: likelihood of the hypothesis; Δ lnL: difference in likelihood between the evaluated alternative hypothesis (H1 to H10) and the best ML tree as best explanation of the data (H0). All intergeneric relationships are unmodified compared to the best ML tree, except for the tested hypothesis (shown in shaded areas): **A**: Evaluation of the Hypostomini as sister group of the Ancistrini (sensu Armbruster, 2004a, 2008; H1); **B**: Evaluation of *P. genisetiger* as a member of *Lithoxancistrus* (sensu Isbrücker, 2001; H2); **C**: Evaluation of *P. genisetiger* as a member of the Hypostomini (sensu Heitmans *et al.*, 1983; H7); **D**: Evaluation of the hypothesis proposed by Armbruster (2004a; H4); **E**: Evaluation of *Guyanancistrus* (sensu Heitmans *et al.*, 1983; H7); **F**: Exclusion of *Guyanancistrus* from *Pseudancistrus sensu lato* (sensu Isbrücker, 2001; H6); **G**: Evaluation of *Guyanancistrus* representatives as members of *Lasiancistrus* (sensu Heitmans *et al.*, 1983; H7); **H**: *P. ornoco* constitutes the sister group of the true *Pseudancistrus* within the remaining *Pseudancistrus sensu lato* (H8); **I**: Exclusion of *P. ornoco* from *Pseudancistrus sensu lato*, *Pseudancistrus* from the Orinoco constitutes the sister group of the true *Pseudancistrus* (H9); **J**: *Pseudancistrus* is restricted to the *P. barbatus* group (sensu de Chambrier and Montoya-Burgos, 2008) and the species from Orinoco form the sister group of *Lithoxancistrus* (H10). In hypotheses D to J (H4 to H10) *P. genisetiger* is excluded from *Pseudancistrus sensu lato* due to its phylogenetic position and its exclusion from the Hypostomini (H3).

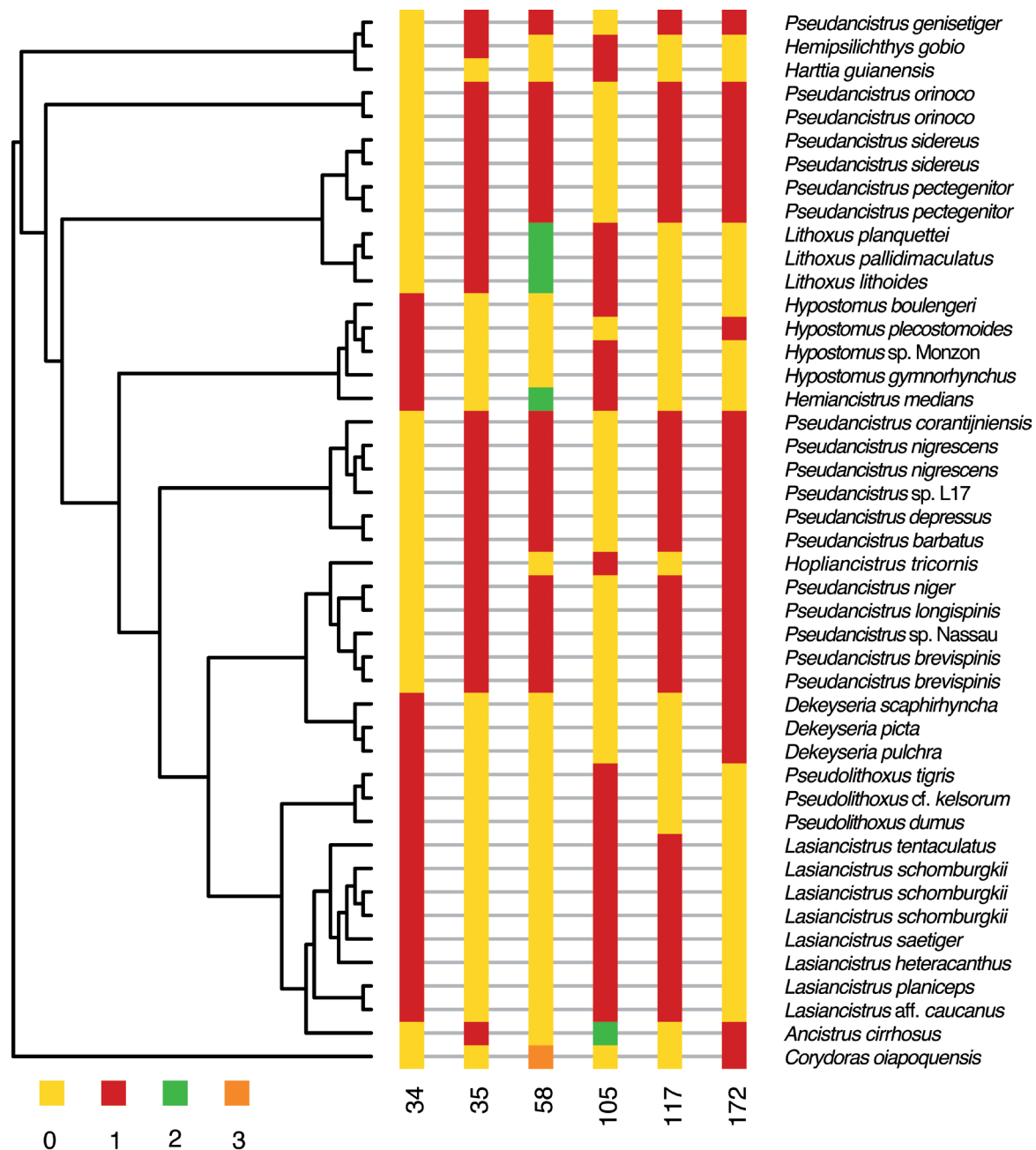


Figure 4. - Distribution of the different character states of the six synapomorphies provided for *Pseudancistrus sensu lato* by Armbruster (2004a, 2008) given the molecular phylogeny. 34: presence or absence of a suture between the pterotic-supracleithrum and hyomandibular; 35: presence or absence of a contact between the hyomandibular and prootic; 58: characteristics of the anterior process of the metapterygoid; 105: size of the nasal bone; 117: presence or absence of an external contact between sphenotic and posteriormost infraorbital; 172: size of the ventral ridge of the basipterygium.

posteriormost infraorbital (117; RUNS-mean = 10.111; $p(X^* \leq X_{obs.}) = 0.0001$), and the size of the ventral ridge of the basipterygium (172; RUNS-mean = 10.341; $p(X^* \leq X_{obs.}) = 0.0001$), displayed accordingly a significant autocorrelation to the molecular phylogeny. The different character states of each variable (Fig. 4) were thus not distributed at random along the phylogeny.

DISCUSSION

One of the strengths of probabilistic methods in phylogenetic analyses is that they allow the comparison of different competing hypotheses for a given data set using the likelihood scores in a well-defined statistical frame (Delsuc and Douzery, 2004). The first approach was developed by

Kishino and Hasegawa (1989) for the comparison of two *a priori* defined topologies in the explanation of the data. However, the frequent incorrect usage of this test consisting of a comparison between the best ML tree (thus an *a posteriori* hypothesis) and an alternative topology, violated the assumptions of the test ($L_{ML} - L_{HI} = 0$). Indeed, the difference in likelihood between the best ML tree and an alternative topology cannot be null (Goldman *et al.*, 2000). To account for this trouble Shimodaira and Hasegawa (1999) developed the SH test and extended it to the simultaneous comparison of multiple topologies. Doing so, the SH test became more conservative to account for multiple testing, and thus more sensitive to type II error when the number of comparisons increases. The SH test therefore provides a very conservative approach often leading to the retention of false hypotheses. To correct for this behaviour of the test, different alternatives (particularly the AU test) were developed (for a review see Shimodaira, 2002).

All phylogenetic reconstructions recovered *Pseudancistrus sensu lato* as paraphyletic. Moreover, the presence of species expected to be our outgroup within the ingroup (and the converse example of *P. genisetiger*) provides strong evidence that the systematics of *Pseudancistrus*, and more generally of the Hypostominae, should be revised in depth using molecular data. Indeed, as an example, the general appearance of *P. genisetiger* (see holotype ANSP 69441 photographed by F. Mendonça available online at the All Catfish Species Inventory Image Base; Morris, Jager and Sabaj Pérez, 2006; http://acsi.acnatsci.org/base/image_show_wrapper.html?target=133448, accessed on the 7th Oct. 2011) and its extremely divergent sequences are more reminiscent of what can be observed in Delturinae rather than Hypostominae, which was supported by the phylogenetic reconstructions. Moreover, the observed paraphyly of Hypostomini (here represented by *Hypostomus*) and Ancistrini within Hypostominae has also been highlighted in previous molecular studies. Montoya-Burgos *et al.* (1997, 1998) based on mitochondrial ribosomal genes, and on the mitochondrial D-loop (Montoya-Burgos *et al.*, 2002), also recovered polyphyletic Ancistrini and Hypostomini. These authors hypothesised that the evertible cheek plates with hypertrophied odontodes, historically diagnostic of the Ancistrini, most probably arose only once in the evolutionary history of the Loricariidae, but were subsequently lost independently in different lineages. More recently, Cramer *et al.* (2011) using other markers (mt COI and nuclear RAG1 and RAG2) in a study focusing on Neoplecostominae and Hypoptopomatinae, but including 25 Hypostominae, also recovered Ancistrini and Hypostomini as paraphyletic.

Following the interpretation of Montoya-Burgos *et al.* (1997, 1998, 2002) concerning the presence or absence of evertible odontodes, the mosaic-like patterns observed in the distribution of the different character states of the six

synapomorphies provided for *Pseudancistrus sensu lato* could be the result of a single ancestral gain followed by subsequent independent losses in different lineages. Conversely, these features could have been gained and lost successively in different lineages. Homoplasy is generally put forward to explain such patterns. Homoplastic character states refer to evolutionary convergences, parallelisms or reversals that are generally randomly distributed along a phylogeny. Such characters are generally analogous and independent of the phylogeny (not inherited from a common ancestor). In a study conducted on the Loricariinae, Covain *et al.* (2008) demonstrated that homoplastic characters, such as the presence or absence of an abdominal cover, the presence or absence of a rostrum, and head shape were independent of the phylogeny and accordingly displayed no phylogenetic autocorrelation. The fact that strong phylogenetic dependence was detected in the distribution of the character states of the six synapomorphies defining *Pseudancistrus sensu lato* favours the retention of homologous ancestral character states in several distinct lineages.

The enforced monophyly of the genus following Armbruster (2004a, 2008) is here significantly rejected, except by the very conservative SH test (see above for an explanation). Monophyly of *Pseudancistrus sensu stricto* (Armbruster and Taphorn, 2008), including, among others, *P. barbatus*, *P. depressus*, *P. nigrescens*, but also *P. genisetiger*, was also significantly rejected. On the contrary, monophyly of the *Pseudancistrus barbatus* group (the true *Pseudancistrus*) formed by the type species, *P. corantijnensis*, *P. depressus* and *P. nigrescens* (de Chambrier and Montoya-Burgos, 2008) is confirmed, with the addition of an undescribed species from the Rio Xingú (*Pseudancistrus* sp. L17). Moreover, *Pseudancistrus* can be here restricted to this lineage because of the significant rejection of all hypotheses implying a sister relationship between this lineage and other lineages of *Pseudancistrus sensu lato*. It therefore appears clear that the older definition of *Pseudancistrus* better reflects the natural grouping of species than the enlarged definition, even when restricted to the seven species considered as *Pseudancistrus sensu stricto*. Species of the *Pseudancistrus barbatus* group all have hypertrophied odontodes along the sides of the snout regardless of sex and season even if greatly enlarged in some males (Darwin, 1871; Isbrücker *et al.*, 1988; Armbruster, 2004b) and lack evertible cheek plates supporting hypertrophied odontodes, distinguishing them easily from all other hypostomins. *Pseudancistrus guentheri* Regan, 1904 and the recently described *P. kwinti* Willink *et al.*, 2010 may be added to this group of true *Pseudancistrus*.

The exclusion from *Pseudancistrus sensu lato* of lineages corresponding to previously described genera such as *Guyanancistrus* and *Lithoxancistrus* is strongly supported by the data, thus sustaining the validity of these different genera. Indeed, initially described as *Lasiancistrus* mem-

bers (Heitmans *et al.*, 1983) and even *Hemiancistrus* for *G. niger* (Norman, 1926), *Guyanancistrus* comprises Guianese species distributed mostly in coastal drainages including *G. brevispinis* (type species), *G. longispinis*, *G. niger* and *Guyanancistrus* sp. Nassau (from the Nassau mountains), and is here revalidated. *Lithoxancistrus* should be also revalidated since the sister relationship between the true *Pseudancistrus* and *L. orinoco* is also significantly rejected. Moreover, *L. orinoco* forms in both phylogenetic reconstructions, and given our sampling, the sister group of all Hypostominae, a position strongly supported by both bootstrap and Bayesian posterior probabilities (Fig. 2). The placement of *P. genisetiger*, previously included in *Lithoxancistrus* by Isbrücker (2001), within Delturinae definitely excludes it from *Lithoxancistrus*. Based on these phylogenetic reconstructions, *Lithoxancistrus* appears to be properly restricted to its type species. However, the inability of the data to reject the hypothesis of a sister relationship between *L. orinoco* and the Orinoco species of *Pseudancistrus* (*P. pectegenitor* and *P. sidereus*) does not provide a clear delineation of *Lithoxancistrus*. Generic limits and definition await further studies that must include the Venezuelan *P. coquenani* Steindachner, 1915 and *P. yekuana* Lujan *et al.*, 2007: taxa that share with *L. orinoco* and *P. pectegenitor* the large dentary papillae that were originally diagnostic for *Lithoxancistrus*. In addition, *L. orinoco* may represent a junior synonym of *P. coquenani* (N.K. Lujan, pers. com.) further rendering the taxonomy confusing and justifying an in depth revision of the genus.

Guyanancistrus has apparently no unique character to distinguish it from other hypostomins. Its original diagnosis by Isbrücker (*in Isbrücker et al.*, 2001) distinguishes it from *Lasiancistrus* based on the characteristic whiskerlike cheek odontodes of the later. Apart from a largely similar appearance and head shape, with a large, rounded and laterally flattened snout, no character seems to unite them. *Guyanancistrus brevispinis*, its type species, lacks the elongated snout odontodes of *Pseudancistrus*, and has evertible cheek plates with hypertrophied odontodes. *Guyanancistrus longispinis*, with longer cheek odontodes and a very long pectoral-fin spine, and *Guyanancistrus* sp. are similar to this, but *G. niger*, also with a very long pectoral spine, has a small area on each side of the snout with a tuft of slightly enlarged odontodes. This feature can be compared to the condition observed in the monotypic *Hopliancistrus tricornis* Isbrücker & Nijssen, 1989 which is here found to be the sister genus of *Guyanancistrus*. Despite the absence of clear variation in morphology, *Guyanancistrus brevispinis* appears to be highly diversified based on the study of Cardoso and Montoya-Burgos (2009), which recovered six allopatric lineages, including five in coastal rivers of Suriname and French Guiana and one in Guianese headwaters of Amazonian Paru de Oeste and Jari Rivers. Other congeneric species are restricted to one river basin, *G. longispinis* and

G. niger in the Oyapock and *Guyanancistrus* sp. Nassau in the Maroni River drainages. *Guyanancistrus brevispinis* is also the most common and abundant species and is regularly collected in both small forest creeks and in the strong current of larger rivers, where it co-occurs with *G. longispinis* and *G. niger*.

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REFERENCES

- ABOUHEIF E., 1999. - A method for testing the assumption of phylogenetic independence in comparative data. *Evol. Ecol. Res.*, 1: 895-909.
- ALEXANDROU M.A., OLIVEIRA C., MAILLARD M., MCGILL R.A.R., NEWTON J., CREER S. & TAYLOR M.I., 2011. - Competition and phylogeny determine community structure in Müllerian co-mimics. *Nature*, 469: 84-89.
- ARMBRUSTER J.W., 2004a. - Phylogenetic relationships of the suckermouth armoured catfishes (Loricariidae) with emphasis on the Hypostominae and Ancistrinae. *Zool. J. Linn. Soc.*, 141: 1-80.
- ARMBRUSTER J.W., 2004b. - *Pseudancistrus sidereus*, a new species from southern Venezuela (Siluriformes: Loricariidae) with a redescription of *Pseudancistrus*. *Zootaxa*, 628: 1-15.
- ARMBRUSTER J.W., 2008. - The genus *Peckoltia* with the description of two new species and a reanalysis of the phylogeny of the genera of the Hypostominae (Siluriformes: Loricariidae). *Zootaxa*, 1822: 1-76.

- ARMBRUSTER J.W. & TAPHORN D.C., 2008. - A new species of *Pseudancistrus* from the Río Caroní, Venezuela (Siluriformes: Loricariidae). *Zootaxa*, 1731: 33-41.
- BLEEKER P., 1862-1863. - Atlas ichthyologique des Indes orientales néerlandaises, publié sous les auspices du gouvernement colonial néerlandais. Tome II.- Siluroïdes, Characoïdes et Hétérobranchoïdes. 112 p., 152 pls. Amsterdam: Fr. Müller.
- CARDOSO Y.P. & MONTÓYA-BURGOS J.I., 2009. - Unexpected diversity in the catfish *Pseudancistrus brevispinis* reveals dispersal routes in a Neotropical center of endemism: the Guyanas Region. *Mol. Ecol.*, 18: 947-964.
- CHIACHIO M.C., OLIVEIRA C. & MONTÓYA-BURGOS J.I., 2008. - Molecular systematic and historical biogeography of the armored Neotropical catfishes Hypoptopomatinae and Neoplecostominae (Siluriformes: Loricariidae). *Mol. Phylogenet. Evol.*, 49: 606-617.
- COVAIN R., DRAY S., FISCH-MULLER S. & MONTÓYA-BURGOS J., 2008. - Assessing phylogenetic dependence of morphological traits using co-inertia prior to investigate character evolution in Loricariinae catfishes. *Mol. Phylogenet. Evol.*, 46: 986-1002.
- CRAMER C.A., BONATTO S.L. & REIS R.E., 2011. - Molecular phylogeny of the Neoplecostominae and Hypoptopomatinae (Siluriformes: Loricariidae) using multiple genes. *Mol. Phylogenet. Evol.*, 59(1): 43-52.
- DARWIN C., 1871. - The Descent of Man, and Selection in Relation to Sex. 507 p. London: John Murray.
- DE CHAMBRIER S. & MONTÓYA-BURGOS J.I., 2008. - *Pseudancistrus corantijnensis*, a new species from the Guyana Shield (Siluriformes: Loricariidae) with a molecular and morphological description of the *Pseudancistrus barbatus* group. *Zootaxa*, 1918: 45-58.
- DELSUC F. & DOUZERY E.J.P., 2004. - Les méthodes probabilistes en phylogénie moléculaire. (1) Les modèles d'évolution des séquences et le maximum de vraisemblance. In: Biosystème 22 : Avenir et Pertinence des Méthodes d'Analyses en Phylogénie moléculaire (Cibois A., Bourgoin T. & Silvain J.-F., eds), pp. 59-74. Paris, France: SFS.
- EFRON B., 1979. - Bootstrap methods: Another look at the jack-knife. *Ann. Statist.*, 7: 1-26.
- FELSENSTEIN J., 1981. - Evolutionary trees from gene frequencies and quantitative characters: Finding maximum likelihood estimates. *Evolution*, 35: 1229-1242.
- FELSENSTEIN J., 1985. - Confidence limits on phylogenies: an approach using the bootstrap. *Evolution*, 39: 783-791.
- FISCH-MULLER S., 2003. - Subfamily Ancistrinae (Armored catfishes). In: Check List of the Freshwater Fishes of South and Central America (Reis R.E., Kullander S.O. & Ferraris C.J. Jr, eds), pp. 373-400. Porto Alegre: Edipucrs.
- FISCH-MULLER S., MONTÓYA-BURGOS J.I., LE BAIL P.Y. & COVAIN R., 2012. - Diversity of the Ancistrini (Siluriformes: Loricariidae) from the Guianas: the *Panaque* group, a molecular appraisal with descriptions of new species. *Cybium*, 36(1): 163-193.
- FRICKE R. & ESCHMEYER W. N., 2010. - A Guide to Fish Collections in the Catalog of Fishes Database. On-line version of 15 January 2010.
- GELMAN A. & RUBIN D.B., 1992. - Inference from iterative simulation using multiple sequences. *Stat. Sci.*, 7(4): 457-511.
- GOLDMAN N., ANDERSON J.P. & RODRIGO A.G., 2000. - Likelihood-based tests of topologies in phylogenetics. *Syst. Biol.*, 49(4): 652-670.
- HALL T.A., 1999. - BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucl. Acids. Symp. Ser.*, 41: 95-98.
- HEITMANS W.R.B., NIJSSEN H. & ISBRÜCKER I.J.H., 1983. - The mailed catfish genus *Lasiancistrus* Regan, 1904, from French Guiana and Surinam, with description of two new species (Pisces, Siluriformes, Loricariidae). *Bijdr. Dierk.*, 53(1): 33-48.
- HUELSENBECK J.P. & RONQUIST F., 2001. - MRBAYES: Bayesian inference of phylogeny. *Bioinformatics*, 17: 754-755.
- ISBRÜCKER I.J.H., 1979. - Les poissons de la famille des Loricariidae, ou poissons-chats cuirassés. *Rev. Fr. Aquariol.*, 6(4): 109-124.
- ISBRÜCKER I.J.H., 1980. - Classification and catalogue of the mailed Loricariidae (Pisces, Siluriformes). *Versl. Tec. Geg., Inst. Taxon. Zööl. (Zööl. Mus.)*, 22: 1-181.
- ISBRÜCKER I.J.H., 2001. - Nomenklator der Gattungen und Arten der Harnischwelse, Familie Loricariidae Rafinesque, 1815 (Teleostei, Ostariophysi). In: Harnischwelse 2 (Stawikowski R., ed.), pp. 25-32. Stuttgart: Die Aquarien- und Terrarienzeitschrift, Sonderheft, Eugen Ulmer.
- ISBRÜCKER I.J.H., NIJSSEN H. & CALA P., 1988. - *Lithoxancistrus orinoco*, nouveau genre et espèce de poissons-chat cuirassé du Rio Orinoco, en Colombie (Pisces, Siluriformes, Loricariidae). *Rev. Fr. Aquariol.*, 15(1): 13-16.
- ISBRÜCKER I.J.H., SEIDEL I., MICHELS J.P., SCHRAMLE E. & WERNER A., 2001. - Diagnose vierzehn neuer Gattungen der Familie Loricariidae Rafinesque, 1815 (Teleostei, Ostariophysi). In: Harnischwelse 2 (Stawikowski R., ed.), pp. 17-24. Stuttgart: Die Aquarien- und Terrarienzeitschrift, Sonderheft, Eugen Ulmer.
- JOBB G., VON HAESELER A. & STRIMMER K., 2004. - TREEFINDER: a powerful graphical analysis environment for molecular phylogenetics. *BMC Evol. Biol.*, 4: 18.
- KISHINO H. & HASEGAWA M., 1989. - Evaluation of the maximum likelihood estimate of the evolutionary tree topologies from DNA sequence data, and the branching order in Hominoidea. *J. Mol. Evol.*, 29: 170-179.
- KISHINO H., MIYATA T. & HASEGAWA M., 1990. - Maximum likelihood inference of protein phylogeny and the origin of chloroplasts. *J. Mol. Evol.*, 31: 151-160.
- KOCHER T.D., THOMAS W.K., MEYER A., EDWARDS S.V., PÄÄBO S., VILLABLANCA F.X. & WILSON W.C., 1989. - Dynamics of mitochondrial DNA evolution in animals: amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA*, 86: 6196-6200.
- KUMAR S., SKJÆVELAND Å., ORR R.J.S., ENGER P., RUDEN, T., MEVIK B.H., BURKI F., BOTNEN A. & SHALCHIAN-TABRIZI K., 2009. - AIR: A batch-oriented web program package for construction of supermatrices ready for phylogenomic analyses. *BMC Bioinf.*, 10: 357.
- LE BAIL P.Y., KEITH P. & PLANQUETTE P., 2000. - Atlas des Poissons d'eau douce de Guyane. Tome 2, fascicule II: Siluriformes. 307 p. *Patrimoines Nat.*, 43(2). Paris: MNHN/SPN.
- LEGENDRE P. & LAPOINTE F.J., 2004. - Assessing congruence among distance matrices: single malt Scotch whiskies revisited. *Aust. N. Z. J. Stat.*, 46: 615-629.
- LUJAN N.K. & ARMBRUSTER J.W., 2011. - The Guiana Shield. In: Historical Biogeography of Neotropical Freshwater Fishes (Albert J. & Reis R.E., eds), pp. 211-224. Berkeley: Univ. of California Press.

- LUJAN N.K., ARMBRUSTER J.W. & SABAJ M.H., 2007. - Two new species of *Pseudancistrus* from southern Venezuela. *Ichthyol. Explor. Freshw.*, 18(2): 163-174.
- MANTEL N., 1967. - The detection of disease clustering and a generalized regression approach. *Cancer Res.*, 27: 209-220.
- MEYER A., 1993. - Evolution of mitochondrial DNA in fishes. In: *Biochemistry and Molecular Biology of Fishes*. Vol. 2: Molecular Biology Frontiers (Hochachka P.W. & Mommsen P., eds), pp. 1-38. Amsterdam, the Netherlands: Elsevier Science.
- MONTOYA-BURGOS J.I., MULLER S., WEBER C. & PAWLOWSKI J., 1997. - Phylogenetic relationships between Hypostominae and Ancistrinae (Siluroidei: Loricariidae): First results from mitochondrial 12S and 16S rRNA gene sequences. *Rev. Suisse Zool.*, 104(1): 185-198.
- MONTOYA-BURGOS J.I., MULLER S., WEBER C. & PAWLOWSKI J., 1998. - Phylogenetic relationships of the Loricariidae (Siluriformes) based on mitochondrial rRNA gene sequences. In: *Phylogeny and Classification of Neotropical Fishes. Part 3: Siluriformes* (Malabarba L.R., Reis R.E., Vari R.P., Lucena Z.M.S. & Lucena C.A.S., eds), pp. 363-374. Porto Alegre, Brasil: Edipucrs.
- MONTOYA-BURGOS J.I., WEBER C. & LE BAIL P.Y., 2002. - Phylogenetic relationships within *Hypostomus* (Siluriformes: Loricariidae) and related genera based on mitochondrial D-loop sequences. *Rev. Suisse Zool.*, 109(2): 369-382.
- MORRIS P.J., YAGER H.M. (progs) & SABAJ PÉREZ M.H. (ed.), 2006. - ACSImagebase: A digital archive of catfish images compiled by participants in the All Catfish Species Inventory. [WWW image Database]. <http://acsi.acnatsci.org/base>.
- NORMAN J.R., 1926. - Description of nine new fresh-water fishes from French Guiana and Brazil. *Ann. Mag. Nat. Hist.*, (ser. 99) 18(103): 91-97.
- OUBOTER P.E. & MOL J.H.A., 1993. - The fish fauna of Suriname. In: *The Freshwater Ecosystems of Suriname* (Ouboter P.E., ed.), pp. 133-313. Dordrecht: Kluwer Academic Publishers.
- PARADIS E., 2006. - Analysis of Phylogenetics and Evolution with R. 211 p. New York: Springer.
- PARADIS E., CLAUDE J. & STRIMMER K., 2004. - APE: analyses of phylogenetics and evolution in R language. *Bioinformatics*, 20: 289-290.
- R DEVELOPMENT CORE TEAM, 2009. - R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. ISBN 3-900051-07-0. <http://www.R-project.org>.
- RAMBAUT A. & DRUMMOND A.J., 2007. - Tracer v1.4, Available from <http://beast.bio.ed.ac.uk/Tracer>.
- REEVE J. & ABOUHEIF E., 2003. - Phylogenetic Independence Version 2.0. Distributed freely by the authors on request. Department of Biology, McGill Univ.
- RODRIGUEZ M.S., ORTEGA H. & COVAIN R., 2011. - Inter-generic phylogenetic relationships in Loricariinae catfishes (Siluriformes: Loricariidae), with description of *Fonchiiloricaria nanodon*: a new genus and species from Peru. *J. Fish Biol.*, 79: 875-895.
- RONQUIST F. & HUELSENBECK J.P., 2003. - MRBAYES 3: Bayesian phylogenetic inference under mixed models. *Bioinformatics*, 19: 1572-1574.
- SCHAEFER S.A., 1986. - Historical biology of the loricariid catfishes: Phylogenetics and functional morphology. PhD thesis, 198 p. Univ. of Chicago, USA.
- SHIMODAIRA H., 2002. - An approximately unbiased test of phylogenetic tree selection. *Syst. Biol.*, 51: 492-508.
- SHIMODAIRA H. & HASEGAWA M., 1999. - Multiple comparisons of log-likelihoods with applications to phylogenetic inference. *Mol. Biol. Evol.*, 16: 1114-1116.
- SOKAL R. & ROHLF F.J., 1995. - Biometry. The principles and practice of statistics in biological research. 3rd edit., 887 p. New York: W. H. Freeman and Company.
- STRIMMER K. & RAMBAUT A., 2002. - Inferring confidence sets of possibly misspecified gene trees. *Proc. R. Soc. B.*, 269:137-142.
- SUGIURA N., 1978. - Further analysis of the data by Akaike's information criterion and the finite corrections. *Commun. Stat.-Theor. M.*, A7: 13-26.
- TAVARÉ S., 1986. - Some probabilistic and statistical problems in the analysis of DNA sequences. In: *Some mathematical questions in biology - DNA sequence analysis* (Miura R.M., ed.), pp. 57-86. Providence: American Math. Soc.
- THOMPSON J.D., HIGGINS D.G. & GIBSON T.J., 1994. - CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.*, 22(22): 4673-4680.
- WILLINK P.W., MOL J.H. & CHERNOFF B., 2010. - A new species of suckermouth armored catfish, *Pseudancistrus kwinti* (Siluriformes: Loricariidae) from the Coppename River drainage, Central Suriname Nature Reserve, Suriname. *Zootaxa*, 2332: 40-48.